All that glitters may not be gold, but at least it contains free electrons.
John Desmond Baernal, Irish physicist (1901–1971)

CHEMICAL EXPOSURES

Cancer and TCDD: The Mitochondrial Connection

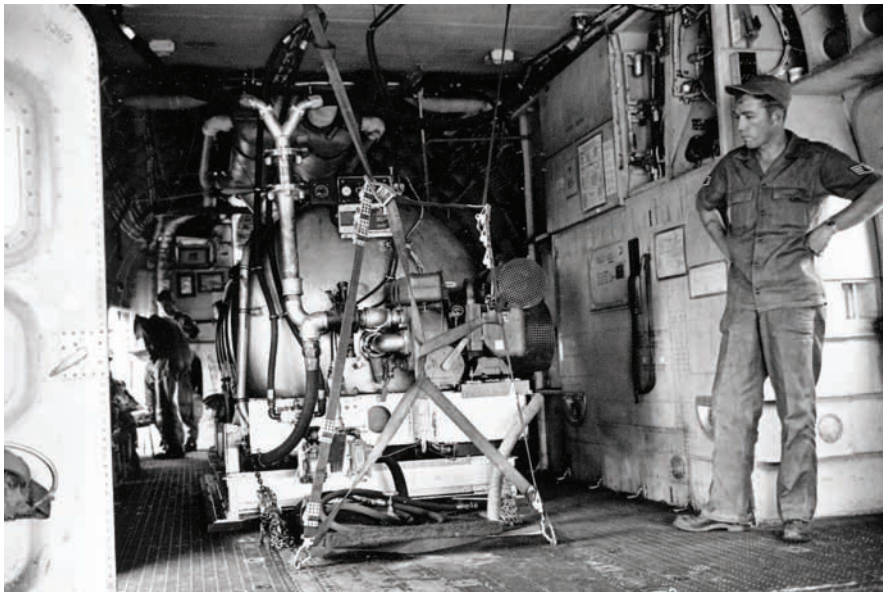
During the Vietnam War, from 1961 to 1971, U.S. military forces sprayed millions of gallons of the herbicide Agent Orange over vast tracts of Southeast Asian jungle, mainly in an effort to remove foliage and expose enemy troops. Troops were exposed to TCDD that contaminated the Agent Orange, and since the 1970s, elevated blood TCDD concentrations have been implicated in many cancers, skin rashes, and other health problems experienced by Vietnam veterans. Although TCDD is carcinogenic, it is not directly genotoxic. A report in the 8 January 2008 *Proceedings of the National Academy of Sciences* now demonstrates one of the ways that TCDD may promote cancer's growth and spread.

The new study describes a novel mechanism of TCDD action that focuses on the mitochondria: "We found that TCDD induces tumor cell proliferation and invasion by directly acting on mitochondrial transcription machinery and inducing mitochondrial respiratory stress," says principal investigator Narayan G. Avadhani, a biochemistry professor at the University of Pennsylvania. Such mitochondrial dysfunction inhibits apoptosis in malignant cells and increases the invasive potential of cancer. Mitochondrial dysfunction is also associated with conditions such as heart disease, diabetes, obesity, blindness, deafness, kidney disease, and neurodegenerative disorders, as well as with aging.

"[The respiratory stress-signaling] cascade culminates in the activation of a large number of nuclear genes that affect various cellular processes including cell metabolism, proliferation, and apoptosis," says lead author Gopa Biswas, a researcher in Avadhani's lab. "We have now established that TCDD alters cellular morphology and physiology through a similar mechanism."

It is generally accepted that adverse effects of TCDD result from its activation of the Ah receptor, with effects occurring at very low exposures. In the presence of TCDD, the Ah receptor has been shown to either induce or suppress the transcription of numerous genes that have been linked with cancer development via changes in tumor suppressor proteins, oncogenes, growth factors, and cell cycle proteins, among other factors.

Mitochondrial dysfunction may entail a more fundamental mechanism. It appears that TCDD-induced mitochondrial stress signaling in cancer cells is propagated in part through the Ah receptor but also acts through mechanisms that are independent of the Ah receptor, such as by inducing protein kinase C and extracellular signal–regulated kinases.



A U.S. Air Force crew member stands by a defoliant mixing machine inside one of three C-123 aircraft used to clear jungle growth in South Vietnam; August 1963.

AP Photo/Horst Faas

"Our findings show that at subtoxic levels of ten to fifty nanomolar, TCDD is sufficient to cause mitochondrial dysfunction and induce the signaling cascade," says Avadhani. "These results raise concerns over the adverse health implications of dioxins and PCBs even at very low levels."

In both animal and human studies (notably epidemiologic analyses of cancer rates following the 1976 industrial accident in Seveso, Italy), TCDD exposure has increased cancer incidence and mortality at all cancer sites rather than at a few specific sites. In 1997, the International Agency for Research on Cancer upgraded TCDD to a Group 1 human carcinogen on the basis of mechanistic data. Considering subsequent dose–response assessments for TCDD and cancer, Kyle Steenland, a professor of environmental and occupational health at Emory University, and colleagues argued in the September 2004 issue of *EHP* that "TCDD exposure levels close to those in the general population may be carcinogenic and argue for caution in setting the upper ranges of long-term permissible exposure to dioxins."

The present study is limited in that it involved skeletal myoblasts, not living organisms. "These findings are significant but unfortunately provide no *in vivo* data showing tumor progression in animals due to loss of mitochondrial function by TCDD," says Keshav K. Singh, a cancer geneticist at Roswell Park Cancer Institute in Buffalo, New York. "At a minimum, xenograft studies in mice are needed." Avadhani now plans to study the precise mitochondrial targets of different polychlorinated biphenyls (a related group of compounds) that lead to reduced mitochondrial transcription and then examine the implications of this pathway in tumor progression *in vivo*. He sees possible implications for the prevention of breast, pancreatic, and other endocrine cancers.

Recognition that the carcinogenic effects of environmental toxicants may originate in disruption of mitochondrial biology could prove important for the future development of cancer prevention and treatment procedures related to TCDD and other dioxin exposures. "The new findings suggest that the risk of cancer may be reduced by avoiding or lowering exposure to environmental mitochondrial toxicants as well as [possibly] by optimizing mitochondrial energy metabolism by nutritional and medicinal means," says Egil Fosslien, a pathology professor emeritus at the University of Illinois at Chicago. **–M. Nathaniel Mead**

ALTERNATIVE TEST MODELS

Toxicity Testing Takes Stock

Early February 2008 saw the celebration of the first 10 years of work by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the federally funded body charged with promoting the regulatory acceptance of scientifically valid safety testing methods that replace, reduce, or refine the use of animals. ICCVAM also released a five-year plan establishing priorities for research, translation, and validation activities. The plan was unveiled during an anniversary symposium held 5 February 2008 in Bethesda, Maryland.

ICCVAM conducts technical evaluations of alternative testing methods proposed for regulatory use, makes recommendations to regulatory agencies on the usefulness and limitations of new methods, identifies knowledge and data gaps that need to be addressed with further research and development efforts, and coordinates with similar efforts internationally. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), housed at the NIEHS, provides administrative, operational, and scientific support to the interagency committee.

To date, ICCVAM's recommendations have resulted in national and international acceptance of alternatives for acute oral toxicity, skin corrosivity, and allergic contact dermatitis, three of the most common types of toxicity assays. ICCVAM has also recommended use of the Bovine Corneal Opacity and Permeability and Isolated Chicken Eye tests to assess eye irritation; regulatory decisions on these assays are due in April 2008.

William Stokes, director of NICEATM, says the tests reviewed by ICCVAM are essential to translating research findings from bench to bedside. "Our 'bedside' is more effective public health prevention measures," he says. "The improved alternatives recommended by ICCVAM help prevent disease and injury so you don't end up in the bed in the first place."

"ICCVAM is essential," says John Bailey, executive vice president for science at the Personal Care Products Council (formerly the Cosmetics, Toiletry, and Fragrance Association). "It brings the science together, and allows a transparent assessment."

ICCVAM's new five-year plan lists four areas of emphasis: establishing priorities for future testing; identifying and encouraging appropriate research efforts; educating stakeholders about the acceptance and appropriate use of improved methods; and improving partnerships with and between those inside and outside ICCVAM. Some of the approaches emphasized for further development include high-throughput assays, use of species such as roundworms and tadpoles, and better biomarkers of toxic effects.

Despite the committee's successes, ICCVAM's efforts have received mixed reviews. Jessica Sandler, director of the Regulatory Testing Division for People for the Ethical Treatment of Animals, who strongly supported ICCVAM when it was formed, says progress has been painfully slow. She attributes this in part to ICCVAM not taking advantage of the work of sister organizations such as the European Centre for the Validation of Alternative Methods (ECVAM), which has endorsed the use of many more alternative test methods than its U.S. counterpart.

But Stokes explains that ICCVAM, NICEATM, and ECVAM in fact worked very closely in recent years. ICCVAM's activities are limited to the review of test methods applicable to regulatory testing, while ECVAM has a broader mandate to address animal use for all areas of research and testing (e.g., screening to prioritize chemicals for product development). Stokes notes that ECVAM is a center with numerous laboratories and a large full-time staff, whereas ICCVAM is a committee with no laboratories. ICCVAM must also operate with a high level of transparency and the opportunity for broad public and stakeholder input in contrast to ECVAM's less-extensive closed review process. Moreover, Stokes says, many of the assays recommended by ECVAM have not been accepted by European regulatory agencies.

Daniel Krewski, chairman of a National Research Council committee that produced the 12 June 2007 report *Toxicity Testing in the 21st Century*, affirms the value of ICCVAM's work so far. However, the committee recommends that an entirely different federal framework, possibly with a size and budget akin to that of the National Toxicology Program, be rapidly adopted to overcome ICCVAM's limitations. Such a program would be "quite a paradigm shift from what we're doing now," he says, but ICCVAM could be viewed as a transitional step toward this new direction.

Meanwhile, the new five-year plan may help expedite improvements, says Sonya Lunder, a senior analyst with the Environmental Working Group. She has many concerns about the ICCVAM review process but appreciates the plan: "It gives us a benchmark to measure progress." The plan is available at http://iccvam.niehs.nih.gov/docs/5yearplan.htm. **–Bob Weinhold**

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Savory Relief for Arsenic Poisoning?

In the February 2008 issue of *Food and Chemical Toxicology*, researchers from India report that aqueous garlic extract (AGE) fed to at-risk individuals may reduce the toxic effects of arsenic. Rats receiving daily doses of arsenic equivalent to the levels in groundwater from heavily arsenic-contaminated areas of the Bengal Basin retained significantly less of the element in blood and liver and excreted significantly more in urine when fed 2 mg/mL AGE. The researchers believe the antioxidant properties of garlic, along with the chelating efficacy exhibited, led to the success of the treatment. AGE was also seen to significantly reduce intracellular reactive oxygen species in several cell types.

In Utero Cigarette Smoke Exposure and Age at Menopause

It is well documented that women who smoke cigarettes begin menopause earlier than nonsmokers. Using data from a U.S. national study on the health effects of prenatal diethylstilbestrol exposure, a team of researchers reported in the 11 January 2008 advance access edition of the *American Journal of Epidemiology* that study participants who had never smoked cigarettes but had been prenatally exposed to maternal cigarette smoke experienced earlier-onset menopause. Moreover, previously noted associations between current smoking and age at menopause were not observed among these women.

Antibiotic Resistance Seen in Arctic Wildfowl

Swedish researchers reported in the January 2008 issue of *Emerging Infectious Diseases* that birds living in three different geographic regions of the Arctic tundra carry *E. coli* bacteria resistant to multiple types of antibiotics. These birds, which lived in northeastern Siberia, northern Alaska, and northern Greenland, are believed to have had no contact with humans. The researchers proposed three possible explanations for their findings: the birds could have been exposed to the bacteria through contact with other species of birds migrating from other regions, or resistance could have developed either through spontaneous mutations or through horizontal gene transfer from other microbes.

The western sandpiper, one of the Arctic species studied

Cardiovascular Health

Hard Data for Hard Water

Are people who drink "hard" water containing higher levels of calcium and/or magnesium less likely to suffer cardiovascular disease? This is the question that delegates who attended a World Health Organization (WHO) meeting 21–22 January 2008 in Geneva, Switzerland, are now trying to answer once and for all.

The idea that hard water—particularly that with higher magnesium concentrations—helps ward off cardiovascular problems has been around for 50 years. However, due to the ecologic nature of most studies, uncontrolled confounding factors, and the different variables and outcomes measured, no firm conclusions have ever been drawn. The WHO is therefore coordinating worldwide efforts to compare cardiovascular morbidity before and after changes in the calcium/magnesium content of water supplies.

The aim of the Geneva meeting was to discuss how such a study—ultimately a composite of many smaller studies from different nations—should be performed. "A prospective, multi-country study following a single protocol would be the best way to ensure a sufficiently large sample for overall analysis . . . if we are to make meaningful comparisons," says Paul Hunter, a professor of health protection at the University of East Anglia, United Kingdom, whose group has been testing a possible protocol.

Hunter's work involved obtaining mortality and residence data on individuals in areas where notable changes in water hardness had occurred through the introduction or cessation of softening practices, allowing trends in cardiovascular mortality before and after the change in water hardness to be detected.

Controlling the confounding factors in a final meta-analysis involving populations from different countries could pose problems, but "the 'before and after' nature of the individual studies should certainly provide meaningful results at the population level," he says.

The mechanism by which hard water may provide protection against cardiovascular disease remains a matter of debate. The extra calcium it carries could help reduce blood pressure, whereas low serum magnesium concentrations—common to people living in soft-water areas—appear linked with arrhythmias. "Couple this with the fact that many of today's refined foods are low in magnesium, that many people in developed countries either do not cover or only barely cover their magnesium needs, and that magnesium in drinking water is more bioavailable than that in food, and you can see how [even the relatively small] extra supply of this mineral to people in hard-water areas could be beneficial," says Frantisek Kozísek, head of the National Reference Centre for Drinking Water in Prague, Czech Republic. "Cooking food in soft water also tends to remove magnesium, calcium, and other essential elements from food, making matters worse."

The results could lead to countries adopting legislation to supplement drinking water supplies in soft-water areas with calcium and magnesium. Kozísek has already proposed that levels of calcium and magnesium in drinking water be set at 40–80 mg/L and 20–30 mg/L, respectively. "The available evidence suggests these ranges could be beneficial, and . . . there is no evidence that harder water causes any harm," he explains.

Regu Regunathan, a consultant for the Water Quality Association, says that any recommendations on magnesium or other minerals must be based on absolutely solid data; otherwise, desalination plants and industries providing water softeners and reverse osmosis devices could be needlessly affected. Indeed, soft water has palpable technical advantages over hard water, including reduced scaling in appliances, pipes, and on surfaces, as well as better soap lathering. To this, Kozísek responds, "If health and technical aspects of water are in contradiction, then cost–benefit analyses of the consequences of both aspects should be made to decide what is more important for society." **–Adrian Burton**

Cancer

Dairy Paradox

The etiology of colorectal disease revolves around genetic and environmental factors, particularly diet. A meta-analysis in the 7 July 2004 issue of the *Journal of the National Cancer Institute* suggested that consuming more dairy products and calcium may reduce colorectal cancer risk, but epidemiologic studies on this link have yielded inconsistent results. One explanation for this inconsistency may be the timing of exposure: cancer develops over decades, and early-life exposures to carcinogens and growth factors could be a critical factor. A new study designed to address this possibility has found that adults who consumed more dairy during childhood may have a greater risk of developing colorectal cancer in adulthood. The results appear in the December 2007 issue of the *American Journal of Clinical Nutrition*.

Colorectal cancer is among the leading causes of mortality in developed countries; according to the National Cancer Institute, about 630,000 deaths were expected to occur worldwide in 2007. Even moderate changes in diet and lifestyle could prevent at least 70% of all colorectal cancer cases, according to a review in the December 2002 *Gastroenterology Clinics of North America*. The main culprits appear to be excessive caloric intake, as well as frequent consumption of red meat, processed meats, alcohol, and refined carbohydrates.

The historical cohort study employed data from the Carnegie Survey, conceptualized by Sir John Boyd Orr, which recorded food consumption patterns in 1,343 English and Scottish families from 1937 to 1939. The survey was designed to investigate the long-range impact of children's diet, growth, living conditions, and health on adult cardiovascular disease.

Dietary data were obtained using a 7-day household inventory; also, weighed inventories of all foods in the household were conducted at the start and end of the survey period. The average follow-up time for adults included in this study was 65 years; 4,374 individuals were available for follow-up between 1948 and 2005. Daily intake of dairy products ranged from less than 0.5 cup at the lowest level to nearly 2 cups at the highest; liquid milk constituted 94% of the dairy intake.

Those individuals who grew up in families reporting the highest levels of dairy consumption showed a nearly threefold increase in the risk of colorectal cancer compared with those from families reporting the lowest intake. The elevated risk remained even after the researchers adjusted the data for potential confounders such as socioeconomic status and meat, fruit, and vegetable intake. No other cancers were significantly affected by higher dairy intakes.

"The mechanisms underlying these associations remain unknown, but there is increasing evidence that nutrition early in life can have long-lasting programming effects," says lead author Jolieke C. van der Pols, an epidemiologist at the University of Queensland. For example, childhood dairy intake appears to be inversely associated with adulthood concentrations of insulin-like growth

Joseph Tart/EHP/istock

ehpnet European Chemicals Agency

On 1 June 2007, Europe's new legislation for managing chemicals, REACH (Regulation, Evaluation, Authorisation and Restriction of Chemicals), entered into force. Under the auspices of REACH, the European Chemicals Agency (ECHA) was set up. ECHA's scope involves implementing and managing the technical, scientific, and administrative work of REACH as well as working to ensure consistency among the European Union (EU) countries with regard to the legislation. The Helsinki-based agency has established a website at http://echa.europa.eu/ to provide a central online source for news and information about its work.

The ECHA website has links to information for industry, policy makers, and the general public about the scope of REACH. The REACH page on the website takes visitors to the REACH Navigator, an interactive tool that allows companies to learn more about their responsibilities regarding REACH compliance. Companies find out what they need to do by answering a series of questions posed by the Navigator. The Frequently Asked Questions section addresses general topics as well as questions about specific classes of chemicals. The Guidance section of the REACH page covers the technical aspects of the legislation. Included here are documents explaining the various REACH processes, including registration, data sharing, classification of chemicals, and preparation of chemical safety reports.

The Software Tools page of the ECHA website provides information on REACH-IT and IUCLID 5. REACH-IT allows companies to set up webpages through which they can submit their chemical registration information. Personnel from ECHA and EU member states can use these pages to review this information. REACH-IT also provides a means for companies that manufacture the same chemicals to contact one another, whereas members of the public can use it to find out information on the types of chemicals that are produced in the EU. IUCLID 5 is the International Uniform Chemical Information Database, which companies can use to store and report information on the properties of chemicals. IUCLID 5 uses data-gathering templates developed by the Organisation for Economic Co-operation and Development. **–Erin E. Dooley**

factor 1 (IGF-1), a key player in the development and progression of colorectal cancer. But it's the effect of early dairy intake on childhood (rather than on adult) concentrations of IGF-1 that may be the important mediator of colorectal cancer risk.

The findings seem perplexing given previous research showing an association between high dairy/calcium consumption and lower risk of colorectal cancer in adulthood. "In adults, this protection occurs despite the increase in growth factors, which would be expected to increase risk," says epidemiologist Edward Giovannucci of the Harvard School of Public Health. "It is possible, though not proven, that the increase in growth factors early in life may be more important for colorectal cancer risk." Giovannucci asserts that the new findings are biologically plausible and warrant efforts to replicate these findings in other populations and settings.

Andrew Szilagyi, a gastroenterologist and assistant professor of medicine at McGill University School of Medicine, points out the lack of data on adult dietary intakes in the Boyd Orr cohort. "We do not know any aspects of dietary intake in adulthood," Szilagyi says. "Nor do we know that the adults who developed colorectal cancer were also the very children in the families that indeed had higher dairy intakes." In light of the fact that most studies have reported a protective effect of dairy products, it is important to determine the extent to which the former diet was continued into adulthood, he notes.

The milk consumption levels identified as posing a significant cancer risk in the Boyd Orr cohort are similar to current average intakes for U.S. children. Nonetheless, the researchers assert that it would be premature to consider altering current guidelines for children's nutrition. "Dairy products are important contributors to children's intake of protein, vitamins, and minerals," says van der Pols. "Because this is only the first study to show associations between childhood dairy consumption and risk of cancer in adulthood, more evidence is needed before any firm conclusions can be drawn." **–M. Nathaniel Mead**

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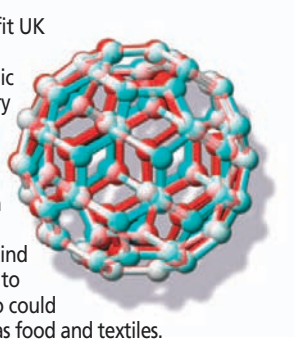
UK Organic Certifier Says No to Nanomaterials



The Soil Association, a nonprofit UK organization that certifies the majority of that nation's organic products, announced in January 2008 it will no longer allow man-made nanomaterials (materials with a mean size of less than 200 nm) to be used in goods carrying its certification label. This rule, the first of its kind in the world, will apply mainly to personal care products but also could apply to other categories such as food and textiles. The group cites insufficient evidence on the impact of nanomaterials on the environment and human health.

China Begins National Survey of Pollution Sources

Although China has set goals to cut emissions of major pollutants, emissions of sulfur dioxide and some indicators of poor water quality increased in 2006. Experts have decried the lack of trustworthy statistics on the sources and extent of pollution and the number of remediation facilities. In response, the Chinese government invested US$100 million in 2007 to launch the collection of data from industrial, agricultural, and residential sources at sites throughout China, beginning in February 2008. The work, which is being overseen by the State Environmental Protection Administration and the Ministry of Agriculture, will also compile information on methods of environmental remediation currently available in the country. Data collection is projected to be complete by mid-2008.

Ancient Chinese Cancer Secret?

Researchers at the University of Texas M.D. Anderson Cancer Center are currently conducting clinical trials in Shanghai on a cancer therapy that uses the venom of the Asiatic toad (*Bufo gargarizans*). China's use of the venom in the treatment of a number of illnesses can be traced back to the tenth century. In the clinical trials, compounds from the venom have proven beneficial and caused no apparent side effects in patients with advanced liver, pancreatic, or

Bufo gargarizans

lung cancer. In mouse studies, the compound was better at shrinking pancreatic tumors than gemcitabine, a standard chemotherapy drug. Cardiac glycosides in the venom are thought to inhibit proteins that promote cancer cell growth, thereby causing cancer cell death. Although potentially toxic at high doses, these glycosides are used to treat congestive heart failure. Currently, the venom-based treatment is administered by injection, but the Texas researchers hope to develop a pill form.